

## STUDIES ON THE PLANT CELL.—VI.

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### SECTION V. CELL ACTIVITIES AT CRITICAL PERIODS OF ONTOGENY IN PLANTS.

WE shall discuss in this paper the behavior of the protoplasm at a number of critical periods in the life history of plants when the organism passes from one phase to another of a fundamentally different character. At such times great changes take place in the potentialities of the cells which inaugurate the new developments, changes that are generally most conspicuously shown in the structure of the nucleus. Some of the most interesting events of cell and nuclear history take place at these times, as would be expected from the importance of the phenomena. We shall treat the material under the following heads: (1) Gametogenesis, (2) Fertilization, (3) Sporogenesis, (4) Reduction of the Chromosomes, (5) Apogamy, (6) Apospory, (7) Hybridization, (8) Xenia.

#### I. GAMETOGENESIS.

The events of gametogenesis are clearly known for the higher plants but there is some confusion and almost no detailed information in the accounts of the thallophytes where the nuclei are very small and the details of the mitoses preceding the formation of sexual cells exceedingly difficult of study.

There is complete agreement among all investigators that the mitoses which precede the differentiation of gamete nuclei in spermatophytes, pteridophytes, and bryophytes are typical karyokinetic figures not differing essentially in the behavior of the chromosomes from the mitoses generally characteristic of the gametophyte generation. This information is based upon a

large number of studies of nuclear figures in antheridia and archegonia, the generative cell of the pollen tube and micropylar region of the embryo-sac. There are no reduction phenomena in these higher groups at the period of gametogenesis.

The subject is complicated in some types of spermatophytes where the gametophyte phase is so reduced that the mitoses which precede gametogenesis may follow immediately upon the two mitoses characteristic of sporogenesis or be separated from them by only one or two divisions. For example, it is known in several types of the lily family (*Lilium*, *Tulipa*, *Fritillaria*, *Erythronium*, etc.) that the two mitoses of sporogenesis (heterotypic and homotypic) are included in the embryo-sac and become a part of that gametophyte history. The third and final mitosis in this history differentiates the egg in the micropylar end of the embryo-sac and is a typical nuclear division. This subject was treated in some detail in Section III of these "Studies" (*Amer. Nat.*, vol. 38, pp. 741-745, 1904). When the mitoses of sporogenesis are not included within the embryo-sac we find almost without exception three typical mitoses preceding the differentiation of the egg in the angiosperms and a very large number in the gymnosperms, and of course in the pteridophytes and bryophytes the whole vegetative period of the gametophyte which is generally green and self-supporting. There are from two to three mitoses in the pollen grain and male gametophyte of the angiosperms before the development of the sperm nuclei and a somewhat larger and more variable number among the gymnosperms. It is necessary at the outset to understand clearly what are the events of gametogenesis in spermatophytes because several authors have carried the phenomena of sporogenesis over into the period of gametogenesis, where it can have no proper place in exact morphology. Such papers will be treated in connection with "Sporogenesis" and "Reduction of the Chromosomes," for they concern primarily these phenomena alone.

Gametogenesis must be considered at present chiefly from our knowledge of the conditions in the higher plants as they furnish almost the only detailed information that we have on the subject. Upon this as a basis we are justified in suggesting

possibilities in the thallophytes which must remain as speculations until investigations have advanced much farther in this difficult field of cell study. The basis of any theories at present must be phylogenetic, a principle that has not been followed in some of the work upon the thallophytes.

Gametogenesis in plants is full of interest because of the sharp differences from the processes of spermatogenesis and oögenesis in animals. In animals the period of gametogenesis is one of unusual activity. After the germ cells are differentiated there follows a period of cell growth, with the peculiar activity termed synapsis, during which the number of chromosomes is reduced to one half the number characteristic of the species. The germ cells emerge from the growth periods as primary spermatocytes or oöcytes which give rise respectively by two successive mitoses to four spermatids or to an egg with its accompanying polar bodies. The gametes have one half the number of chromosomes characteristic of the species, so that the period of gametogenesis is one of chromosome reduction. The character of this process of reduction will be considered when we take up the analogous phenomena in plants after the discussion of sporogenesis. Gametogenesis in plants is in striking contrast to that in animals. In all higher groups (those above the thallophytes) we know that the gametes have the same number of chromosomes as the vegetative cells of the parent plant (gametophyte). There is no reduction of the chromosomes at the time of gametogenesis, that phenomenon taking place at the end of the sporophyte generation with sporogenesis. Also, there are no peculiarities of the mitoses immediately preceding gametogenesis excepting such as concern the development of cilia-bearing organs (blepharoplasts) or slight peculiarities in the form or size of the spindles, for such nuclear figures are frequently different in these particulars from the mitoses in vegetative cells of the gametophyte. The differences concern chiefly the structure of the sperm, and have been described in our account of that structure (*Amer. Nat.*, vol. 38, July and August, p. 576, 1904).

To Strasburger above all others should be given the credit of making clear these important characteristics of gametogene-

sis in plants. Strasburger's paper of 1894 on "The Periodic Reduction of the Number of Chromosomes in the Life History of Living Organisms" (*Annals of Bot.*, vol. 8, p. 281) was the first elaborate presentation of the principles of gametogenesis and reduction phenomena in plants and has become classical as the foundation of the present attitude in botanical science and the basis and stimulus of a large amount of confirmatory research. The matter really crystallized after the discovery that the sporophyte generation of the higher plants possessed nuclei with twice the number of chromosomes characteristic of the gametophyte and that the reduction took place in the spore mother-cell just previous to sporogenesis.

These facts were gradually established by a number of investigations beginning with Strasburger ('84, '88) and Guignard ('84, '85). Guignard ('91) presented the first complete count of the number of chromosomes in the life history of a plant (*Lilium martagon*), determining the reduction period to be in the spore mother-cell, and Overton ('93 a and b) independently reached the same conclusions for the same plant and extended the knowledge of the chromosome count in gametophyte and sporophyte to a number of other types. Overton's paper was important in its suggestiveness for extended research among the higher cryptogams. Other investigations followed shortly in the gymnosperms, pteridophytes, and liverworts, all supporting the view that the nuclei of the sporophyte generation, following the fusion of gamete nuclei, had double the number of chromosomes characteristic of the gametophyte and that the reduction phenomena occurred at the end of the sporophyte generation in the spore mother-cell. The significance of reduction phenomena at sporogenesis must be phylogenetic since it represents a return of the organism at this time to the ancestral gametophyte condition. The details of this literature belong to the account of "Sporogenesis" and "Reduction of the Chromosomes," and will be taken up later. But it is necessary to present the outline at this time to make clear the important fact that no reduction of the chromosomes takes place during gametogenesis in all groups above the thallophytes.

The theories of gametogenesis among the thallophytes rest

upon information which in point of completeness falls very far short of our knowledge of the groups above. Indeed, no forms have been studied with the detail that is known in higher groups chiefly for the reason that the investigator is forced to deal with very small nuclei and mitotic figures whose chromosomes are exceedingly minute and because of various technical difficulties. The theories in general fall into two groups: (1) those which have an obvious basis in attempts to reconcile events with the processes of gametogenesis in animals, and (2) those proceeding from the view that for phylogenetic reasons the periods and phenomena of gametogenesis in the lower plants should correspond with those of the higher.

We may pass over with a few words the early crude attempts to establish structures for plants comparable to the polar bodies of animals. For example at the conclusion of oögenesis in some algæ (*e. g.*, *Vaucheria*, *Edogonium*) a globule of slime is exuded with the opening of the oögonium. It was suggested that such material is thrown off from the egg but we now know that it is not protoplasmic in character but is apparently derived from a softening of the cell wall. Then the ventral canal cell has been compared to a polar body but it seems clear now that all of the canal cells are homologous and a part of what was formerly an extensive gametogenous tissue within the archegonium. Then the small group of cells cut off below the oögonium of the Charales and the fragmented nuclear material in the trichogyne of the red algæ have been compared to substance thrown off from the egg but without any knowledge of the nuclear structure. Finally the nuclear degeneration which is a very conspicuous feature of oögenesis in certain groups whose oögonia are multinucleate (*Peronosporales*, *Saprolegniales*, *Pelvetia*, etc.) has been considered related to reduction phenomena. But the nuclei in all of these forms bear every evidence of being in each type homologous structures whose large numbers have a phylogenetic *raison d'être* and the extensive degeneration is associated with the principles of sexual evolution which tend to conserve protoplasm for the good of a lesser number of gamete nuclei even to the sacrifice of others that are potentially equivalent.

We will now consider the few instances among the thallo-

phytes in which a reduction of the chromosomes is reported just previous to or during gametogenesis. The best known case is *Fucus* since this type has been studied by three investigators: Farmer and Williams ('98) and Strasburger ('97a). They agree in describing the nuclear figure that differentiates the oögonium from the stalk cell as exhibiting a large number of chromosomes (28 or 30) while the three mitoses within the oögonium, which give rise to the eight eggs, present only one half that number (14 or 15). Apparently there is a reduction by one half just before the mitoses in the oögonium. Since there is no sporophyte generation in *Fucus* it is of course difficult to compare these conditions with those in higher plants, but, as will be explained later, there are some reasons why we should not expect to find reduction phenomena at gametogenesis in any thallophyte.

Reduction phenomena at gametogenesis have also been suggested for various types of the Peronosporales and Saprolegniales but not, however, in exactly the same way as in *Fucus*. There are always, as far as is known, one or two mitoses within the oögonium before the gamete nuclei are organized and it has been held that these are reduction divisions by Rosenberg for the Peronosporales and by Trow for the Saprolegniales. Rosenberg (:03b) described for the oögonium of *Plasmopara* a condition of synapsis in the nuclei preceding the two mitoses and compared this sequence with the events of sporogenesis in higher plants in which the two divisions within the spore mother-cell are preceded by a period of synapsis. Rosenberg did not determine the number of chromosomes in the vegetative nuclei so that he has no positive evidence of reduction in the oögonia. With respect to the two mitoses and the preliminary synapsis I have already pointed out in criticism of Rosenberg's studies (*Bot. Gaz.*, vol. 36, p. 154, 1903) that the number of mitoses is variable in the oögonia of the Peronosporales and Saprolegniales and apparently entirely absent in the species of *Vaucheria* studied by myself (Davis, :04a). Also, the phenomenon of synapsis, which is easily recognized in the large nuclei of the spore mother-cell, would be difficult to establish in the small nuclei within the oögonia of the forms mentioned above. Nuclei can be found

in a number of structures with their contents somewhat massed at one side or in the center but such conditions must not be confused with the remarkable process of synapsis in the spore mother-cell. Among all the excellent studies of gametogenesis in the Peronosporales I cannot find any clear evidence of a reduction of the chromosomes at gametogenesis.

Quite different is the account that Trow (:04) brings forward to support his view of chromosome reduction during gametogenesis in the Saprolegniales. Trow describes two mitoses in the oögonium of *Achlya debaryana*: in the first the number of chromosomes is eight which becomes reduced to four in the second. Trow's account of a second mitosis in *Achlya* is very different in a number of particulars from the results of all investigations on gametogenesis in the Peronosporales and Saprolegniales. Two centrosomes with radiations are said to appear at the poles of the spindle at anaphase, structures which were not present in the first mitosis. Some of these asters become the center of the egg origins and are later accompanied by deeply staining material constituting a body which Trow terms an ovocentrum and which perhaps corresponds to a cœnocentrum. Relatively few of the nuclei in the oögonium are said to pass through this second mitosis and some of their products, with the accompanying asters, break down. The remainder become the functional gamete nuclei of the eggs. There are many complex activities described by Trow in connection with the appearance of the asters during the second mitosis and also at the side of the sperm nuclei which are said to enter the oögonium, events that cannot be correlated with the processes of gametogenesis and fertilization as we understand them for the Peronosporales. They are treated briefly in a review by myself (*Bot. Gaz.*, vol. 39, p. 61, 1905), where, however, I misunderstood a distinction that Trow draws between the aster and the ovocentrum (see an answer by Trow, *Bot. Gaz.*, vol. 39, p. 300, 1905). My impression is that either Trow has been mistaken in his interpretations or that there are present events which must entirely change our conception of gametogenesis in the Saprolegniales and Peronosporales, but which are not fully explained by Trow's paper.

Let us now think of gametogenesis among the thallophytes

with reference to what we know of the process in higher groups and the principles of the origin and evolution of sex and the sporophyte among the lower. It seems clear that the sporophyte generation is characterized by a double number of chromosomes as a result of the fusion of gamete nuclei at fertilization. We must then lay the fundamental inception or origin of the sporophyte to the stimulus of the sexual act. That is, the sexually formed fusion cell must have different potentialities from the germ plasm of the parent gametophyte and it cannot produce a gametophyte again until these potentialities are worked off and the protoplasm returns to the dead level of the ancestral stock (the gametophyte). By the potentialities of the sporophyte plasm we mean primarily a greater energy or growth stimulus which must express itself differently from the gametophyte. Morphologically we can only distinguish sporophyte plasm from gametophyte plasm by the double number of the chromosomes but of course the complexities of the sexual act would make great differences in the chemical structure of the two. The divergences in the history of the gametophyte and sporophyte, as shown throughout ontogeny and phylogeny, are but the final expressions of the different potentialities of the protoplasm in each generation. The morphological forms of expression of the sporophyte are extraordinarily various and in the long evolutionary history of this generation have developed great structural differentiation but with every life history the sporophyte has the same beginning (fertilization, with the doubling of the chromosomes) and the same ending (sporogenesis, with chromosome reduction). Between the beginning and the end is intercalated a vegetative period, short and simple in some forms, and very long and elaborate in others. The history of the development of this vegetative period or the evolution of the sporophyte is a subject far outside of and secondary to the scope of this discussion. We are only concerned with the protoplasmic activities at the beginning (fertilization) and the end (sporogenesis) of the sporophyte generation.

We know nothing of the behavior of the chromosomes in types of the thallophytes which illustrate most closely our conception of the origin of sex and of the sporophyte generation.



I refer to many lower algæ such as *Ulothrix*, forms of the *Volvocaceæ*, *Œdogonium*, *Coleochæte*, and many others. However, the homologies of primitive gametes and their origin from types of asexual zoöspores is very clear in a number of groups. We can see nothing in the morphology and mode of development of these reproductive cells to suggest reduction phenomena when gametes are produced. The primitive gamete is generally somewhat smaller than its homologue the zoöspore, often because the protoplasm of the gamete mother-cell becomes distributed in a greater number of daughter elements. It is well known that the conditions that lead to conjugation are exceedingly variable, depending upon environmental factors and one often cannot tell at the time whether a swarm spore will show sexual habits or germinate without conjugation. The most satisfactory theory of the origin of sex in plants regards primitive gametes as weaker or lacking in certain potentialities of vegetative growth and the conjugation as a mutually coöperative process resulting in a rejuvenescence of the protoplasm. The fact that many simple types of gametes will germinate without fertilization and produce small and weak sporelings shows that vegetative possibilities are not entirely lost. Investigations on the chromosome history among these forms, difficult though they be, are some of the most interesting subjects of botanical research. We know some general principles of the origin and evolution of sex in plants (Davis, :01b, :03a) but of the chromosome history in the simplest types of gametogenesis nothing is known.

With respect to the history of the chromosomes in the simplest sporophytes we are also as ignorant as in the simplest types of gametogenesis. We have excellent reasons for believing that the sporophyte generation is represented among the thallophytes in a number of very simple conditions. Numbers of zygospores and oöspores (*e. g.*, *Ulothrix*, *Œdogonium*, forms of the *Conjugales* and *Volvocaceæ*, etc.) give rise on germination to several daughter cells. In higher forms this growth period is lengthened to the formation of a reproductive tissue (*Coleochæte*) and in the great groups of the *Rhodophyceæ*, *Ascomycetes*, and *Basidiomycetes* there is present an extensive development from the fertilized female cell (or its equivalent when apogamy obtains)

involving the development of a vegetative structure before the period of sporogenesis. From the studies of Wolfe (:04) we know that the sporophyte portion of *Nemalion* (the cystocarp) contains nuclei with double the number of chromosomes (about 16) present in the gametophyte (about 8) and that the period of chromosome reduction is apparently just previous to the development of the carpospores (sporogenesis). Williams (:04a and b) has recently determined that the asexual plant of *Dictyota* is a sporophyte generation with double the number of chromosomes (32) found in the sexual plant (16). The reduction occurs here during a rather long period of preparation on the part of the nucleus in the tetraspore mother-cell and the reduced number appears in the two mitoses that form the tetraspores. These events closely parallel those in the spore mother-cell of higher plants and will be discussed further under "Sporogenesis."

William's (:04b) account of gametogenesis in *Dictyota* is the most complete that we have for any thallophyte. The oögonia and antheridia are cut off from a stalk cell by a mitosis which presents 16 chromosomes, the number characteristic of the gametophyte. The contents of the oögonium forms a single egg and consequently presents no mitotic phenomena. The antheridium develops over 1500 sperms thus exhibiting a large number of successive divisions. These all show 16 chromosomes and the mitoses are typical, not differing in any essential from the division in the stalk cell. The entire absence of mitoses in the oögonium and the great number in the antheridium are striking facts which show that no especial significance can be attached to nuclear divisions within sexual organs of this type. There is no place for reduction phenomena within these sexual organs and none precede their development.

These studies of Williams and Wolfe justify us in expecting that other thallophytes will support their discoveries that the product of the sexual act will have a fusion nucleus with double the number of chromosomes present in the sexual plant (gametophyte) and that reduction phenomena may be expected to follow the sexual act and not precede it as in animals. In such thallophytes as have no sporophyte generation we may suppose, as Strasburger ('94a) suggested, that the number of chromo-

somes is reduced with the germination of the sexually formed cell so that the protoplasm returns at once to the potentialities of the gametophyte. It is quite possible that the four zoöspores produced from the oöspore of *Ædogonium* and the four nuclei found in the germinating zygosporos of the desmids and *Spirogyra* may indicate divisions concerned with reduction phenomena similar to those in the tetraspore mother-cells of *Dictyota* (which may also be expected in the tetraspore mother-cell of the red algæ) and in the spore mother-cell of the higher plants.

For these reasons we seem to be justified in taking a critical attitude towards the accounts of chromosome reduction at gametogenesis among the thallophytes. The logic of the situation would lead us to expect that every sexual act gives a doubling of the chromosomes and an impulse towards the development of a sporophyte phase in plants which must be worked off before the protoplasm is in condition to reproduce the parent gametophyte. Reduction phenomena should follow then every sexual act. If it takes place immediately with the germination of the sexually formed cell there is of course no sporophyte generation. Because the conception of the sporophyte generation with reduction of the chromosomes at sporogenesis is so clearly established in higher groups, those investigators who claim reduction phenomena at gametogenesis must expect their views to be severely scrutinized and accept the responsibility of presenting very clear and convincing proof of their conclusions. The author does not think that this evidence is supplied in satisfactory form by any investigation so far.

## 2. FERTILIZATION.

In Section IV of these "Studies" we described the most important phenomena of fertilization under the caption "Sexual Cell Unions and Nuclear Fusions." It will not be necessary to discuss the facts of the phenomena in detail again. This account will take up the more theoretical aspects of the events of fertilization and their relation to other critical periods of ontogeny.

Plants are in complete agreement with animals in the follow-

ing chief events and principles of fertilization. Thus Van Beneden's conclusion of 1883 that sexual nuclei are equivalent in their chromatin content at the time of fusion irrespective of differences in size is admirably borne out by Miss Ferguson's (:04) studies on the pine. In this form as in the gymnosperms generally the male nucleus is much smaller than the female and comes to lie in a depression in the latter before the actual fusion takes place. After the fusion the paternal and maternal chromosomes are found in two groups side by side preparatory to the first cleavage mitosis and are indistinguishable except for their position; the chromatin of the two sexes is equal in amount as far as can be seen. Then the observations of the Hertwig brothers, in 1887, and Boveri, in 1889 and 1895, that the sperm nucleus could enter and cause the development of denucleated eggs or their fragments thus taking the part of a female nucleus in parthenogenesis, were established for plants by Winkler's (:01) experiments on *Cystoseira*. Winkler was able to divide the egg of this brown alga into a nucleated and a non-nucleated portion and he found that sperms entered the non-nucleated parts and caused them to develop sporelings side by side with the fertilized nucleated portions. The sporelings from the non-nucleated fragments, controlled by the sperm nuclei alone, developed about half as rapidly as those from the originally nucleated portions which of course were dominated by sexually formed fusion nuclei, but the two sets of sporelings were alike in form as far as they were grown. Only with respect to Boveri's celebrated theory that the sperm brings to the egg in the centrosome the mechanism of cell division, do plants fail to support the conclusions of certain zoölogists with respect to the most important events of fertilization. This point upon which zoölogists are not in full accord will be discussed later. There is general agreement in the view that the male nucleus of plants supplies chromosomes equal in number and equivalent quantitatively to the female, and general accord in the conclusions that the chromosomes by their individuality, apparent permanence of structure, and fixed behavior must be bearers of hereditary characters.

Evidence from the most recent investigations upon favorable forms of both animals and plants indicates that the chromosomes

from both gametes maintain their independence and never fuse at the immediate time of fertilization. We have reason to assume, chiefly from zoölogical studies, that the paternal and maternal chromosomes of plants remain independent throughout the entire sporophyte generation and that no fusion takes place until the period of chromosome reduction at sporogenesis. If no sporophyte generation is present we should expect the fusion and reduction of the chromosomes to occur after the sexually formed cell had passed through a period of rest (for all reduction phenomena seem to require considerable time) unless there be actually such reduction during gametogenesis in the thallophytes as reported for *Fucus* and *Saprolegnia*. The morphology of the chromosomes is probably unchanged by the immediate act of fertilization. The fusion nucleus simply contains double the number of chromosomes present in each gamete nucleus which increases by so much the metabolic possibilities which lie in these structures.

Besides chromatin the sperm brings into the egg a certain amount of cytoplasm. Some of this may be the substance of the blepharoplast or other kinoplasm associated with the nucleus but there is often besides considerable granular trophoplasm, sometimes with inclusions of starch and other food substances, and the male gamete of certain thallophytes contains a chromatophore. There is no reason to suppose that development especially characteristic of fertilization, the sporophyte generation, has any relation to this trophoplasm with its food inclusions, excepting as it may stimulate growth which is to be expected whenever organic food material is introduced into protoplasm. But we can hardly believe that the formative elements or the rudiments of further development especially those of a sporophytic character lie in this region of the protoplasm. They must be sought in the nuclei and in the only stable elements of the nuclei, the chromosomes.

It has been held at times by botanists, following the lead of certain zoölogists, that the sperm or sperm nucleus introduced a centrosome into the egg which organized the first cleavage-spindle and thereby played a necessary part in starting cell division. Such a centrosome would naturally be sought in the

blepharoplast which is clearly analogous to the middle piece of the animal spermatozoon. We have no evidence that such events ever take place in the eggs of plants. On the contrary we know that the first cleavage-spindle in the eggs of spermatophytes develops without centrosomes from a mesh of fibrillæ. Also the blepharoplasts of the gymnosperms *Cycas*, *Zamia*, and *Ginkgo* remain in the cytoplasm at a distance from the fusion nucleus and Shaw's account of the fern, *Onoclea*, indicates that similar conditions obtain there. We know less about the history of the blepharoplasts within the egg of thallophytes where the first cleavage-spindle frequently has very handsome centrospheres and asters (*e. g.*, *Fucus* and *Dictyota*). Strasburger ('97a) pointed out that one of the asters of the first cleavage-spindle in *Fucus* arose near the point where the male nucleus united with the female. However, Farmer and Williams ('98) believe that centrospheres of the first cleavage-spindle in *Fucus* are formed *de novo* and Williams (:04b) came to the same conclusion for *Dictyota*. There are some very interesting features in the comparative study that Williams (:04b) has made on the development of the first segmentation spindle in the fertilized and parthenogenetic eggs of *Dictyota*. The spindle in the parthenogenetic egg is multipolar and develops from an intranuclear kinoplasmic mesh and there are no centrospheres. But in the fertilized egg a centrosphere always appears at the side of the nucleus and apparently divides into two which separate until they lie at opposite poles of the mature spindle. Yet Williams after a very careful study concludes that this centrosphere arises *de novo* and believes that the stimulus of fertilization enables the fusion nucleus to form a centrosphere external to itself, a thing which is not possible for the nucleus of the parthenogenetic egg.

It seems then probable that the only structures of the sperm that preserve their morphological entity in the fertilized eggs of plants are the chromosomes. Whatever may be the relation of the blepharoplast and other cytoplasmic structures as stimuli to the development of the egg they cannot be regarded as fixed factors in the problem of heredity. It is very probable that they introduce valuable food material, perhaps important fer-

ments, substances of great service, although possibly not absolutely necessary to the successive metabolic processes which characterize growth and development. But the fact remains that we have in the chromosomes the only new morphological elements. And the progress of research seems ever to strengthen the general view that in the chromosomes are contained the directive rudiments of development and that they are the bearers of hereditary principles. Nuclear studies on apogamous forms will certainly prove of great interest in this connection. We have reason to expect some very important results from thorough cell studies on apogamy and apospory.

The best developed theory of fertilization in plants is that of Strasburger and a statement of his views should precede any comments of other authors. Strasburger has written much on the phenomena of fertilization; important considerations may be found in his papers of '94a, b, '97c, :00a, b, :01, and :04a. Strasburger points out that the protoplasm of the egg is predominately trophoplasmic in character because of the proportionately very large amount of cytoplasm with granular inclusions that are evidently food material or the products of metabolism. On the other hand the cytoplasm of the sperm contains relatively little trophoplasm and much kinoplasm, especially when the sperm is a ciliated cell with a large blepharoplast. As Strasburger conceives kinoplasm to be the active substance of spindle formation, he concludes that the sperm might bring to the well nourished egg, rich in trophoplasm, the substance necessary to start the mechanism of mitosis. In its broad aspects this view is very similar to the celebrated theory of Boveri, 1887, that the spermatozoon supplied the animal egg with the centrosome which is conceived as necessary to start mitotic processes and that the egg is powerless to divide before fertilization because it lacks such a structure.

Another feature of Strasburger's views (advanced in his paper of :00b) appears to have grown out of the discovery of the so called "double fertilization" in the embryo-sac and other nuclear fusions whose sexual significance is not clear, together with the phenomena of parthenogenesis as produced experimentally in many studies of recent years. Strasburger considers that two

processes are involved in the sexual act. The first, termed "vegetative fertilization," is simply the stimulus to growth which results from the fusion of two nuclei or other masses of protoplasm. The second, called "generative fertilization," involves deeper factors than those of mere growth stimulus. These lie in the union of germ plasm of diverse parentage with the mingling of hereditary racial characters and individual variations and the establishment of a new organism which may have possibilities of development quite different from the parent form. The effects of "vegetative fertilization" may be imparted to protoplasm artificially by chemical and physical stimuli as has been done in the numerous experiments of Klebs and Loeb on the conditions which induce parthenogenetic development. "Generative fertilization" has a phylogenetic significance and a background which is entirely apart from the mere vegetative processes of cell growth and division.

It is apparent that Strasburger's theory is open to the same line of criticism that has been brought against the universal application of Boveri's hypothesis that the spermatozöon brings to the egg the agent of cell division as a centrosome. The investigations of several zoölogists indicate that one or both of the centrosomes in the first cleavage-spindle may be derived from the egg or may be formed *de novo* (see Wilson, :00, pp. 196, 208). The kinoplasm of the plant sperm, whether in the form of a blepharoplast or as an ill defined accompaniment of the sperm nucleus has not been shown to take part in the formation of the first cleavage spindle. There is no evidence that the blepharoplast retains its organic entity in the egg to pass over into a centrosome or centrosphere. Of course the kinoplasm which lies immediately without the nuclear membrane of the sperm, and there is sometimes a conspicuous amount of this densely granular protoplasm, must merge with similar kinoplasm associated with the egg nucleus at the time of fusion. For example Miss Robertson (:04) and Coulter and Land (:05) note in *Torreya* that the sperm nucleus brings to that of the egg a large amount of accompanying kinoplasm which forms an investing layer around the fusion nucleus. It is reasonable to suppose that the mixing of these masses of kinoplasm with the



fusion of the gamete nuclei would give material for a larger and more highly differentiated nuclear figure in the first cleavage of the egg.

Williams' (:04b) observations and conclusions on Dictyota are especially interesting in this connection for he shows that the first cleavage-spindles in the parthenogenetic eggs are intranuclear and multipolar, showing no dominant kinoplasmic centers while the fertilized eggs form each a well differentiated centrosphere with radiations, exterior to the nuclear membrane, which clearly guides the whole process of spindle formation. Williams does not hold that this centrosphere comes as an organized structure from either sperm or egg but is developed *de novo* by the fusion nucleus as the result of the general stimulus of fertilization. The evidence, then, furnished by studies on fertilization in plants, indicates that the chromosomes alone maintain morphological independence throughout the process of fertilization and that the kinoplasmic (archoplasmic) elements play no part in the phenomena as fixed morphological structures but simply contribute their substance to the general union of cytoplasm with cytoplasm, and that any specialized kinoplasmic structures of the first cleavage spindle are formed *de novo*. While it is true that the sperm brings to the egg much kinoplasm it may well be questioned whether such kinoplasm is a necessary factor in the formation of the first cleavage-spindle. It seems more probable that the development of achromatic structures in the first mitosis following fertilization is due rather to the general stimulus of cell and nuclear fusion than to particular structures supplied by either sperm or egg.

The second phase of Strasburger's theory of fertilization concerns a separation of the two processes in the sexual act: (1) the mere growth stimulus, "vegetative fertilization," that may be expected with the union of any two masses of protoplasm, and (2) the clearly defined sexual phenomena, "generative fertilization," which lies in the union of germ plasm of different parentage and diverse potentialities and which leads to the inheritance of these characteristics. It seems clear that the two processes are really present and can be clearly distinguished. But it may be strongly questioned whether the factors charac-

terizing the first (vegetative fertilization) should really be considered a part of the sexual act. Strasburger regards the processes of "generative fertilization" as essential to the sexual act. The growth stimulus "vegetative fertilization" is always to be expected as an accompaniment of fertilization. It may be given to cells in other ways than by the sexual act and is found in cell and nuclear fusions which for phylogenetic reasons are plainly not sexual.

The experimental work of recent years on the conditions determining artificial parthenogenesis have done much to define the sorts of factors which stimulate growth and division of sexual cells when the process of fertilization is suppressed. Klebs for plants and Loeb for animals have been foremost in these studies and they have shown that what seem to be very minor changes in the environment of the sexual cell may suffice to give a gamete the power of immediate development without fertilization. Thus the egg of the sea urchin will develop parthenogenetically to an advanced stage when placed for a short time in sea water containing magnesium chloride and then brought back to normal sea water. Nathansohn (:oo) found that a small proportion (about 7 %) of the eggs of *Marsilia vestita* would germinate parthenogenetically when the megaspores were cultivated for 24 hours at the rather high temperature of 35° C. and then left to continue their development at 27° C. There are then a number of factors such as varying osmotic pressure, temperature, and in some cases chemical reagents which may induce gametes to further development without the usual sexual processes. These reactions seem to be of a similar character to the processes in that phase of sexual reproduction termed "vegetative fertilization" by Strasburger. They give the stimulus to growth but without that essential feature of sexuality, the mingling of germ plasm of different parentage which distinguishes the processes of "generative fertilization."

It seems to the author, for the sake of clearness, that we are trying to include too much under the term fertilization. If the features of "vegetative fertilization," *i. e.*, the growth stimulus, can be introduced experimentally as in artificial parthenogenesis then they cease to be fundamental qualities of the sexual act.

They are accompaniments of sexual processes which may always be expected but nevertheless are not the essential characteristics. The essence of the sexual act (fertilization) is the union of germ plasm with such possibilities of new developments as come from the inheritance of mixed characters from two lines of ancestry. And the more diverse and complex are the characters of the parents the more conspicuous are the essential features of the sexual act. Among lowly organisms and in simpler types of sexual processes the growth stimulus becomes exaggerated in our attention because the features of heredity are not so prominent as in the higher forms. But in the higher groups the varied characters of offspring express clearly the subtle factors concerned with the mingling of diverse germ plasm in the process of fertilization and the growth stimulus recedes into the background.

For these reasons it seems to me that the term fertilization should only be used for the mingling of germ plasm with the possibilities of new combinations in the potentialities of the resulting sexually formed cell and that the growth stimulus should be treated as an accompaniment but quite apart from the essentials of the sexual act. And for these reasons I was careful to include in Section IV under the caption "Sexual Cell Unions and Nuclear Fusions" only illustrations in which the sexual nature of the phenomena was clearly shown by applying a morphological or phylogenetic test to the elements concerned in the process of cell fusion. The phylogenetic test seems to me the only sure way of determining the sexual nature of the members of a cell fusion and there are very few cases in which there can be any hesitation in deciding whether or not such elements are morphologically gametes.

I included under "Asexual Cell Unions and Nuclear Fusions" in Section IV a number of cases in which the sexual nature of the act is under dispute for the reason that none of these satisfy the phylogenetic test. It is perfectly clear that the growth stimulus is a conspicuous feature of these cell and nuclear fusions and that in this feature they resemble sexual processes but this does not, to my mind, make them acts of fertilization or the equivalent of sexual processes. The union of sporidia in the

smuts and of yeast cells, the fusion of nuclei in the teleutospore and basidium and in the apogamous development of ferns, the double fusion of polar nuclei and multiple nuclear fusions in the embryo-sac (*Corydalis*) illustrate phenomena which I cannot regard as sexual even though they have in them elements associated with sexual processes and in certain cases may be substitutes for a former sexual act. In none of these instances can we be positive that the nuclei concerned are morphologically and phylogenetically gamete nuclei. This point was discussed in some detail in Section IV. It seems to me that Blackman's (:04 a, p. 353) conception of the cell fusions preceding the æcidium in *Phragmidium* as "reduced forms of ordinary fertilization" or Farmer's (:03) explanation of apogamy in the fern "as a kind of irregular fertilization" leads to a confusion of a substitute process with a true sexual act. The substitute processes have their true place as phenomena of apogamy. They can, however, only have a sexual significance if they represent the origin of a new set of gametes in the organism, a proposition which is not likely to be maintained by anyone.

### 3. SPOROGENESIS.

We are employing the term sporogenesis, as must have been apparent in preceding divisions of this paper, to designate a characteristic and highly specialized type of spore formation that is universal in all plants above the thallophytes. The process always terminates the sporophyte phase in ontogeny of these higher plants, and is especially distinguished as the period of chromosome reduction in the life history. The cell activities of sporogenesis are therefore of particular interest, and, since spore mother-cells are generally large and their nuclear and cytoplasmic structure especially clearly differentiated, we have perhaps obtained more knowledge of mitotic phenomena from the study of these elements than of any other tissues of the plant body.

The reduction phenomena of sporogenesis have been established in some forms of the thallophytes, certainly in the tetraspore mother-cell of *Dictyota* (Williams, :04a). There are also reasons for suspecting that the oöspore of *Cedogonium* and the

zygospores of Conjugales on germinating present similar events. The teleutospore and basidium are probably also the seat of chromatin reduction (Blackman, :04b) in the formation of spores either directly or through the promycelium. The ascus holds a position at the end of a sporophyte phase which suggests a similar relation in this group of fungi. Chromosome reduction may also be expected in the tetraspore mother-cell of the Rhodophyceæ, as in Dictyota, but this subject has never been investigated. There are occasional red algæ in which the tetraspores are sometimes borne on the same plant with the sexual organs, conditions which may be difficult to explain on the theory that the tetrasporic plant is a sporophyte. Thus *Spermothamnion turneri* on the American coast frequently bears both procarps and tetraspores on the same plant, and I have also seen cystocarpic plants of *Ceramium rubrum* some of whose branches contained tetraspores. Lotsy (:04a) also reports similar conditions in *Chylocladia kaliformis*. The other extremely varied methods of spore formation (zoöspores, conidia, etc.) in the thallophytes do not concern the present discussion. They seem to have no fixed place in the life history and there is nothing to indicate any relation to reduction phenomena, although we actually know nothing about the chromosome history among these lowly forms.

The importance of sporogenesis as a critical period in the life history of higher plants became at once apparent with the discovery that fertilization doubled the number of chromosomes in the nuclei of the sporophyte phase and that the double number was reduced during sporogenesis. As stated in our account of gametogenesis, these facts were first established for a number of spermatophytes by the work of Strasburger ('84, '88, and '94), Guignard ('84, '85, and '91), and Overton ('93 a and b). Guignard ('91) presented for *Lilium martagon* the first complete account of the number of chromosomes in the life history of a plant, and his results were also established independently by Overton ('93 a and b). Then followed confirmatory investigations among the bryophytes in the work of Farmer ('94, '95 a, b, c) and in the pteridophytes by Strasburger ('94, p. 294) for *Osmunda*. Since 1895 the investigations among the spermatophytes have so multiplied that we know the number of chromo-

somes in sporophyte and gametophyte for more than fifty forms. This list may be found in Coulter and Chamberlain's recent text-book, *The Morphology of the Angiosperms*, 1903, p. 81. Farmer's accounts of the number of chromosomes in the Hepaticæ have been confirmed and extended by myself (Davis, '99, :01a) and by Moore (:03). The more recent literature, especially as it concerns the events of spindle formation in the mitoses characteristic of sporogenesis has been treated in our account of the spore mother-cell (*Amer. Nat.*, vol. 38, p. 725, Oct., 1904).

There are two chief periods in the processes of sporogenesis as illustrated in all groups above the thallophytes: (1) a growth period and (2) a period of cell division. In the growth period the spore mother-cells become differentiated from the general sporogenous tissues through a great increase in the amount of protoplasmic material. At some time in this growth period the nucleus of the spore mother-cell exhibits the phenomenon of synapsis, a very characteristic event, recognized by the very much contracted condition of the chromatin network in the interior of the nucleus. Synapsis is believed to hold fundamental relations to reduction phenomena as the time when chromosomes unite with one another in pairs. The period of cell division follows synapsis and is characterized by two mitoses in the spore mother-cell, the second following immediately upon the first, and a segmentation of the protoplasm, sometimes by two successive divisions, and sometimes by a simultaneous cleavage, into four spores. The two mitoses present certain peculiarities in the structure and behavior of their chromosomes which are unlike the events of typical mitoses. The first is known as the heterotypic and the second as the homotypic mitosis. These peculiarities have been recognized for a long time and have furnished the subject of much investigation and contradictory explanations. They were briefly described in Section III (*Amer. Nat.*, vol. 38, p. 740, Oct., 1904) but recent studies of Farmer and Moore (:03, :05) have opened again a discussion which seemed closed at that time. The details of synapsis and the heterotypic and homotypic mitoses will be taken up under the caption, "Reduction of the Chromosomes."

Contrary to a statement in Section III of these studies (*Amer.*

*Nat.*, vol. 38, p. 726, Oct., 1904) there is probably a deep significance in the fact that two mitoses are almost universally present in the spore mother-cell so that four spores are formed. It is probable that these mitoses are always heterotypic and homotypic, although this fact has only been clearly established in comparatively few favorable forms, and that they are indispensable to the mechanism of reduction phenomena. The latest accounts describe the first mitosis as the separation of the two portions of a bivalent chromosome, that is of two chromosomes joined either side by side or end to end, giving it a unique position among the mitoses of the life history. According to these theories the two mitoses of sporogenesis are features of a remarkable mechanism by which the paternal and maternal chromatin after its union in synapsis may become distributed in proportions that can be expressed by mathematical ratios. The peculiarities of the homotypic mitosis depend on a premature fission of the daughter chromosomes of the heterotypic division as will be explained in the next portion of this section. Thus the four spores are the result of these peculiar mitoses and have morphological significance. We are even justified in suspecting that the groups of four spores when found in the thallophytes, as the tetraspores of Dictyota and the red algæ, the four spores formed on the basidium and promycelium and the four spores of nuclei present in the germinating oöspore and zygosporangium of *Cedogonium* and the Conjugales indicate the presence of reduction phenomena simply because the number four is so constant. Williams (: 04a) for Dictyota and Blackman (: 04b) for types of the Uredinales have discovered clear cytological evidence of this reduction phenomenon but we know nothing of the chromosome history in other types.

We have already referred to the fact (Section III, *Amer. Nat.*, vol. 38, p. 743, Oct., 1904), that in the spermatophytes the two mitoses characteristic of sporogenesis are very close to the mitoses which differentiate the gamete nuclei. In the male gametophyte of the Angiosperms there are generally only two mitoses between the events of sporogenesis and gametogenesis and in gymnosperms there is a somewhat larger and variable number. The female gametophyte of the angiosperms usually

presents three mitoses after those of sporogenesis before the egg nucleus is formed. But in a number of types in the lily family (*e. g.*, *Lilium*, *Tulipa*, *Fritillaria*, *Erythronium*, etc.), the mitoses of sporogenesis are actually included in the embryo-sac and the very next mitosis, which is typical, differentiates the egg (see Section III, *Amer. Nat.*, vol. 38, pp. 741-744, Oct., 1904). This is the furthest point attained in the reduction of the gametophyte which in such forms actually includes but a single nuclear division in its history. But however close the mitoses of sporogenesis come to those of gametogenesis it is perfectly clear through the long phylogenetic history in the lower spermatophytes, pteridophytes, and bryophytes that the two are morphologically distinct processes and are always separate. It is unfortunate that the terms spermatogenesis and oögenesis should be applied to processes of sporogenesis as has been done by several authors, for such usage involves a confusion of two events which phylogeny clearly shows to be different in origin and to have back of them the diverging history of sporophyte and gametophyte from the times of thallophyte ancestry, the most remarkable evolutionary history in the plant kingdom.

It is conceivable that some plants may finally reach a stage in their evolutionary history when all the gametophytic mitoses in the pollen grain and embryo-sac will be suppressed and the nuclei resulting from sporogenesis become gamete nuclei. But it is clear that in such an event the gametophyte phase would be obliterated and we should have an entirely new type of life history. There would then be only one organism (derived from the sporophyte) whose gametes would be formed immediately with the differentiation of the pollen grain and embryo-sac. Such an organism would present reduction phenomena with the differentiation of the gametes and its type of life history would be identical with that of animals. We should look for such a reduced life history in groups related to forms in which the mitoses of sporogenesis are included in the embryo-sac and the gametophyte phase is represented by a single nuclear division (*e. g.*, *Lilium*, *Tulipa*, *Fritillaria*, *Erythronium*, etc.). Search among some of the most highly specialized Monocotyledonæ may actually reveal examples of the complete suppression of the female gametophyte.



The speculative possibilities of a suppression of a sexual generation and the assumption of sexuality by an asexual phase were clearly in the mind of Strasburger when he suggested ('94b, p. 852) the possibility that the two mitoses characteristic of oögenesis and spermatogenesis in animals might signify the remains of a former sexual generation now entirely suppressed in the Metazoa. This suggestion was based on the striking similarity of the events of sporogenesis in plants to those of gametogenesis in animals and on the history of sporogenesis as shown in plant phylogeny. This history is remarkably clear and there can be no question but that the phenomena of sporogenesis have developed as the result of sexual processes and are always associated with an asexual generation (sporophyte). It is also clear that the ancestral primitive sexual generation (gametophyte) has steadily degenerated until now it is almost lost in such embryo-sacs as include the two mitoses of sporogenesis within their history. If the sexual generation should become entirely lost the life history of a higher plant would present the same features with respect to the period of chromosome reduction as that of an animal: there would be but one organism, the homologue of the sporophyte which would produce gamete nuclei with reduction phenomena previous to gametogenesis just as in animals. Several authors have expressed views similar to Strasburger's suggestion ('94b, p. 852) or carried the speculation even farther than he. Beard ('95a, p. 444) along these lines of argument combined with conclusions from Bower's ('87) studies on apospory, announced a belief that "Metazoan development was really bound up with an *antithetic* alternation of generations." Lotsy (:05, p. 117) expresses unequivocally the view that the animal body represents an asexual phase (2x generation) and that the sexual phase (x generation) is confined to the sexual cells. Chamberlain (:05) simultaneously with Lotsy and in much greater detail presents a comparison of the phenomena of sporogenesis in plants with gametogenesis in animals tracing the resemblance in the events of chromosome reduction step by step and states his belief that "animals exhibit an alternation of generation comparable with the alternation so well known in plants."

This is not the place to consider this theory in detail from a

zoölogical standpoint since it bears only indirectly upon the material of these papers. Zoölogists have, however, discussed critically Strasburger's suggestions (see Wilson, :00, p. 275, and Häcker, '98, p. 101). The difficulties of accepting this view of a possible *antithetic* alternation of generations in animals seem insurmountable. In the first place there is not the slightest evidence of *antithetic* alternation of generations in the Metazoa or for that matter anywhere in the animal kingdom. The examples of alternation of generations which the zoölogists present among the Coelenterates are all illustrations of *homologous* generations derived from buds. There is no indication of spore formation comparable to the process in the higher plants, so far as I am able to judge, in any group of animals. And also there seems to be accumulating evidence of reduction phenomena previous to the development of sexual cells in the Protozoa which is essentially of the same character as in the Metazoa (see Wilson, :00, pp. 227, 277, and Calkins, :01, p. 233). It is very interesting and remarkable that reduction phenomena should show the same order of events in animals and plants and the facts should be clearly recognized. But I cannot follow those botanists who carry over to the animal kingdom the phylogenetic conclusions which are so clear in plants. The remarkable agreement of the events of sporogenesis in plants with gametogenesis in animals appears to me likely to prove only another illustration of similar biological phenomena which have evolved independently of one another, an illustration comparable with the independent origin of sex, of heterospory, and probably even of the sporophyte generation itself (involving the processes of sporogenesis) in various groups of the plant kingdom.

We have considered this comparison of reduction phenomena in plants with animals chiefly to emphasize the clear cut morphology of the process as understood by the botanist. It does not matter how close the events of sporogenesis may come to those of gametogenesis in the higher angiosperms, the whole background of plant phylogeny, which is wonderfully clear as a whole, shows that reduction phenomena are the product of the asexual generation. It represents, as Strasburger has so well

expressed it ('94a, p. 288), a return on the part of the plant organism in each life history to the condition of an ancestral sexual generation (gametophyte). Reduction phenomena in themselves are not the result of a gradual evolution, whatever may be the complicated history of the sporophyte generation, for they consist always in the sudden reappearance of the primitive number of chromosomes, characteristic of the generation in which sex arose (gametophyte). The cause of reduction phenomena is phylogenetic. The interval that may separate this phenomenon from the responsible sexual act varies immensely in the plant kingdom according to the evolution of the groups concerned. But the suddenness of the appearance of sporogenesis tells in every case the same story of an immediate and total change in the potentialities of the protoplasm in the spore mother-cell, a change which can only be understood as a phylogenetic process deeply seated in the race.

When the events of sporogenesis in plants are considered as processes of spermatogenesis or oögenesis we disregard the most remarkable historic outlines that plant phylogeny can present, to the confusion of clear thought. Botanical science may well be proud of its achievement in outlining with such exactness the relations that the critical periods of gametogenesis, fertilization, and sporogenesis bear to reduction phenomena and too great stress can hardly be laid upon the importance of the results.

#### 4. REDUCTION OF THE CHROMOSOMES.

There are perhaps no activities of the cell which have been the subject of more investigation and discussion than those of chromosome reduction in animals and plants. The reasons are clear. The events of gametogenesis in animals and of sporogenesis in plants have the deepest significance for an understanding of the organization of protoplasm because these are periods when great changes are made evident in the structure of the cells concerned and at the same time in their potentialities. We are forced to conclude that some of the structural changes at least are the cause of the new potentialities and the attempt to establish the cause and effect has been one of the most fruitful and

interesting subjects of cell research. Reduction phenomena also have a deep phylogenetic significance whose history in plants at least can be traced with a remarkable degree of exactness.

We are confident that sporogenesis in plants signifies the sudden return of the organism to the condition of an ancestral sexual generation with the reappearance of a primitive number of chromosomes. The short time consumed in the process and the details and precision of the cell activities show that we are dealing with phenomena whose complicated mechanism can only find explanation in a long phylogenetic history. In the study of reduction phenomena and fertilization we have reached the conclusion that the chromosomes are intimately concerned with the transfer of hereditary qualities and are probably the chief or even the sole bearers of these characters. And thus we enter upon some of the most far reaching problems of biology, those of heredity, hybridization, and the basis for the remarkable ratios of inherited characters which Mendel first clearly set forth.

It seems quite certain for both animals and plants that numerical reduction of the chromosomes takes place through an association of the paternal and maternal chromosomes in pairs to form the reduced number of bivalent chromosomes (dyads). We have presented in Section IV ("Sexual Cell Unions and Nuclear Fusions") the evidence which indicates that paternal and maternal chromosomes do not unite at the immediate time of nuclear fusion in fertilization. On the contrary, in all higher animals and plants the paternal and maternal chromosomes are believed to remain separate throughout the long series of cell divisions in the new generation up to the time of sporogenesis in plants and gametogenesis in animals, both events being characterized by reduction phenomena. The fusion of the chromosomes takes place in the growth period which differentiates the spore mother-cell in plants from the archesporium or the primary gametocyte in animals from the preceding gametogenous tissue. The growth period is one of general protoplasmic accumulation and increase in the chromatin content of the nucleus, and is especially characterized by that peculiar activity in the nucleus termed synapsis. Evidence is accumulating that synapsis is the characteristic

feature of that period when the number of chromosomes is reduced by half.

Synapsis is followed very shortly by the two mitoses characteristic of sporogenesis. These nuclear divisions have given rise to a lengthy literature in which well known investigators have shifted their positions more than once. The discussions have centered on the methods of fission and distribution of the reduced number of bivalent chromosomes which appear in the first mitosis following synapsis. Assuming that the chromatin is organized into smaller units, represented by the chromatin granules (chromomeres, Fol, 1891), which compose the chromosomes, it is at once apparent that these finer elements may become variously distributed according to the structure of the bivalent chromosomes and the character of the mitoses of sporogenesis. Each fusion bivalent chromosome is composed of two chromosomes joined (1) end to end or (2) side by side or (3) it is possible that the chromatin is intricately mixed in the structure. With respect to the mitoses a transverse division of the fusion chromosomes might be expected to give a very different proportionate arrangement of the maternal and paternal chromatin from longitudinal divisions. Should the chromatin granules differ qualitatively from one another then different parts of a chromosome might be expected to have different characteristics which would be distributed by the mitoses of sporogenesis in various proportions or ratios.

It has long been known that the mitoses of sporogenesis present peculiarities in the mode of division and arrangement of the chromosomes at the nuclear plate which make them unlike the typical mitoses of cell division. These peculiarities have led to the designation of the first mitosis as heterotypic and the second as homotypic, terms which are now applied by both botanists and zoölogists although we have now a much more extended knowledge of each type than when Flemming first proposed the classification in 1887. We described the characters of the heterotypic and homotypic mitoses in Section III, "The Spore Mother-cell" (*Amer. Nat.*, vol. 38, p. 740, Oct., 1904), and will presently treat them further since some papers of the past year have opened again a discussion which seemed

closed a few months ago. The chief points of issue in discussions of reduction phenomena have centered around the significance of the heterotypic and homotypic mitoses. A typical mitosis is believed to present merely a quantitative division of each chromosome into two halves equivalent in their potentialities. The evidence for this view lies in the longitudinal fission by which each chromatin granule on the spirem is supposed to divide and contribute half of its substance to each daughter chromosome. Can there be a qualitative division of a chromosome by which one of the parts differs in character from the other, and are there such divisions at the time of sporogenesis in plants and gametogenesis in animals when reduction phenomena take place? These have been the chief topics of dispute in studies of this character for two decades.

The problem then ultimately concerns the structure of the chromosome and the reason for the constant reappearance of the number characteristic of the species at the beginning of each new gametophyte generation. All the prominent theories of heredity assume that the chromosomes are made up of simpler elements which stand for characteristics of the race. These may form various combinations of higher orders and collectively give the qualities of germ plasm. The simplest members that can be observed in such a series of structures are the chromatin granules (chromomeres) which may be found at almost all times in the nucleus and are especially conspicuous when arranged in a row on the linin thread of the spirem. Weismann has developed the most complex conception founded on the above principles and with the most elaborate terminology. Starting with the chromatin granule, which he named an *id*, Weismann assumed that this element is composed of still smaller structures called determinants and biophores, the last being the ultimate living units. Groups of *ids* make up *idants* or chromosomes. The *id* was conceived to possess all the essential characters of the specific germ plasm concerned but *ids* vary somewhat among themselves, determining thus the individual variations of the species. Therefore a chromosome or *idant* will have a varying structure according to the character and distribution of the *ids* which compose it.

When a chromosome divides longitudinally so that each id splits in half, the daughter chromosomes are exactly equivalent and the division of the chromatin is merely quantitative. But should a chromosome divide transversely then two sets of entire ids would be separated from one another and the two daughter chromosomes would differ in proportion as their component ids varied, *i. e.*, the division of the chromatin would be qualitative. These conceptions of the possible structure and mode of division of chromosomes outline the basis of Weismann's theory of heredity and will serve to illustrate the general attitude of those biologists who approach the subject from the standpoint of preformation, although none have cared to formulate such elaborate assumptions as Weismann. However, there is a general agreement among biologists of this school that elements are present in the chromatin which do carry hereditary characters and that the chromatin granule and chromosome have a definite architecture and organic value because of these elements.

Weismann's theory of heredity rests on an interpretation of the complexities of mitosis presented by Roux in 1883. Roux assumed that chromatin was not homogeneous in structure throughout the nucleus, but differed qualitatively in various regions. The elaborate history of mitosis with the formation and division of the chromosomes and their distribution through the mechanism of the spindle seemed inexplicable to Roux except on the theory that portions of the chromatin represented specific characteristics which were sorted and distributed accurately according to some system. There could be no need of such a complicated mechanism as mitosis if the distribution of the chromatin was to be merely quantitative for simple direct nuclear division could perform that operation as effectively as mitosis. Mitosis then became a device for the qualitative distribution of chromatin as well as quantitative and the characters of the daughter cells were determined chiefly by the specific elements which were given to one or the other.

Weismann siezed upon Roux's suggestion of a possible qualitative distribution of chromatin in mitosis and this assumption became a very important feature of his theory of heredity. Weismann postulated two methods of mitosis. By the first the

chromosomes are assumed to split longitudinally into equivalent halves, which are the facts in all vegetative or somatic mitoses so far as is known, and the chromatin is distributed quantitatively. By the second method chromosomes were conceived to split transversely so that one half is carried to each daughter nucleus, and if the two ends of a chromosome differed in the character of their fundamental elements (ids and determinants) the chromatin would be distributed qualitatively. Weismann prophesied in 1887 that this second type of nuclear division (qualitative mitosis) would be found and ever since investigators have steadily searched for a transverse division of the chromosomes. They have been reported in connection with the mitoses of chromosome reduction both for animals and plants and the history of these investigations forms an important part of the subject of reduction phenomena. But the present interpretation of these transverse divisions involves the consideration of factors that were unknown to Weismann and are very different from the significance assigned by him. The effect of Weismann's speculations, as a stimulus to investigations in these lines can, however, hardly be overestimated.

Botanical literature dealing with the two mitoses of sporogenesis presents a confusion of statements respecting the presence or absence of a transverse division of the chromosomes. Strasburger has changed his opinion three times. In his early studies Strasburger ('95) believed that the chromosomes divided longitudinally in both mitoses of sporogenesis. Then, led by studies of Mottier ('97) he concluded ('97b) that the fission of the chromosomes in the second mitosis was transverse. Almost immediately, however, Strasburger and Mottier reverted to the former opinion that the chromosomes divided longitudinally, a view which Strasburger maintained in his lengthy considerations of reduction phenomena in 1900a. Finally in a recent paper (:04b) Strasburger gives a very different interpretation of the events of the first mitosis (heterotypic), based on the study of *Galtonia*, and in general agreement with the most recent conclusions of Farmer and Moore (:03). Farmer ('95b), Farmer and Moore ('95), Miss Sargant ('96, '97), Guignard ('99a), Grégoire ('99), Lloyd (:02), and Mottier have also held that the divisions



of the chromosomes in the mitoses of sporogenesis were longitudinal with somewhat varying views, however, as to the exact time when the two divisions take place. On the other hand Ishikawa ('97), Calkins ('97), Belajeff ('98), and Atkinson ('99, for *Trillium*) have claimed that the second mitosis presented a transverse division. Dixon ('95, '96, :00) and Schaffner ('97) held a position apart from all these investigators, believing, that the chromosomes of the first mitosis of *Lilium* resulted from loops whose free ends became appressed or twisted together finally separating at the angle of the loop and thus constituting a transverse division in this first mitosis. These latter observations accord with the latest conclusions of Farmer and Moore (:03) and Strasburger (:04b). Most of this literature is reviewed in detail in Strasburger's paper of 1900a. We shall omit an historical discussion of this early work for the entire subject is approached from quite a different standpoint in the series of papers which have appeared in the past three years (1903–05) and which give hope of much clearer information on the mitoses of the spore mother-cell.

The remainder of this treatment of "Reduction of the Chromosomes" will take up the recent papers and try to show the drift of the present investigations. These papers had not appeared when the author described the behavior of chromosomes during mitosis in Section II (*Amer. Nat.*, vol. 38, p. 445, June, 1904) and presented the account of the spore mother-cell in Section III (*Amer. Nat.*, vol. 38, pp. 726, 740, Oct., 1904). At that time it seemed probable that Strasburger's conclusions of 1900 held true for all plants, namely, that the chromosomes split longitudinally in both mitoses of sporogenesis as well as in all other mitoses of the life history. Whether these views may have to be materially changed in the light of the most recent work is now a matter of dispute. Yet the ground has shifted so frequently in these perplexing problems that it is hard to feel sanguine of final conclusions even in the hopeful situation of the present. I shall take up the events of sporogenesis in order, beginning with the growth period and synapsis and ending with the two mitoses of the spore mother-cell.

The growth period always extends over a considerable length

of time and may occupy even weeks or months. During this interval the spore mother-cells increase to many times the size of the archesporial cells from which they were derived. There is an immense accumulation of protoplasmic material and a corresponding increase in the size of the nucleus and its chromatin content. The growth may be continued in the spores after the mitoses of sporogenesis, as is characteristically illustrated in the great increase in the size of the megaspores in the pteridophytes and certain embryo-sacs. The most striking nuclear activity of the growth period preceding the mitoses is synapsis. This term is applied to a very characteristic gathering of the chromatin and linin material in a compact tangle or ball at one side of the nucleus and usually near the nucleolus. Nuclei are sometimes in a state of synapsis for several days or perhaps weeks as is shown by the frequency of the stage in sporogenesis. Thus during the entire period of sporogenesis in *Anthoceros* from the inception of the spore mother-cell to the final differentiation of the spores (which must take many days) the period of synapsis occupies from one eighth to one sixth of the entire time (Davis, '99, p. 104). Synapsis has proved to be a very difficult subject for study and few investigators have made detailed observations upon its events. Some have claimed that synapsis is an artifact due either to poor fixation or to a particularly sensitive condition of the cell nucleus by which the chromatin was especially susceptible to shrinkage but it seems certain now that the phenomenon is entirely normal. Miss Sargent ('97, p. 195) has observed synapsis in the living pollen mother-cell of *Lilium martagon*. *Anthoceros* presents a particularly favorable subject for the study of the effects of fixing fluids on spore mother-cells because one may present all stages in the same sporophyte to identical conditions. In a series of experiments on this form (Davis, '99, p. 97) with a number of standard fixing fluids I have always found synapsis at exactly the same period in sporogenesis and at no other time in the process. True synapsis, characteristic of reduction phenomena must be carefully distinguished from other somewhat contracted conditions of the chromatin which are occasionally found in cells. Thus Miyake (*Annals of Bot.*, vol. 17, p. 358, 1903) noted the resemblance to synapsis

of an accumulation of granular material in the nucleus of the central cell of *Picea* and other cases might be cited which superficially resemble synapsis but have no fundamental relation to this peculiar nuclear activity.

Evidence is steadily accumulating that synapsis is a very important period of sporogenesis. Some authors hold, as will be described presently, that it is the time when paternal and maternal chromosomes, which have remained separate throughout the sporophyte generation, become associated in pairs to give the reduced number of the gametophyte. This conclusion makes synapsis the actual period of chromosome reduction and the two succeeding mitoses become merely distributing divisions of the newly formed chromosomes. Montgomery (:01) first suggested for animals that synapsis involved a union of maternal and paternal chromosomes in pairs. Other views, however, regard the reduction of the chromosomes as merely the temporary union of paternal and maternal elements, end to end, to form a bivalent chromosome characteristic of the first or heterotypic mitosis. According to this view the bivalent chromosomes divide transversely so that the halves are distributed as whole chromosomes in the first mitosis.

Two very important papers on reduction phenomena have appeared this year (1905) both of which were preceded by preliminary publications, that of Farmer and Moore (:03) and Allen (:04). These two accounts best represent the attitude of the opposing schools and will be made the chief texts of our treatment. The fundamental points of difference concern the events of synapsis and the heterotypic mitosis while there is complete agreement in the general interpretation of the homotypic mitosis. All authors have reached essentially the same conclusions as regards the purpose and final results of the reduction divisions but the details of the processes of synapsis and the prophase of the heterotypic mitosis are described in radically different ways by various investigators. However, as has been stated, the views fall into two groups or schools, one led by Farmer and Moore with whom Strasburger's recent paper, "Ueber Reduktionsteilung" (:04) expresses essential agreement. The other school includes Allen, Rosenberg, and the

botanists of the Carnoy Institute, Grégoire and Berghs. To the writer the conclusions of the second school seem better founded and we shall present them first. Allen's last paper (:05) gives the most complete statement of their interpretations.

Allen's conclusions (:03, :05) are based on the study of the pollen mother-cell of *Lilium canadense* and his account of synapsis in this form is of great interest for the simplicity of his explanation of the events of this phenomenon and their significance. The nucleus of the young pollen mother-cell following the last mitosis in the archesporium and previous to synapsis contains a network of large irregular masses connected by fibers of varying thickness. The irregular masses, which probably contain both chromatin and linin, are derived from the chromosomes of the previous mitosis but these structures cannot be recognized in the resting nucleus. Nucleoli are present among the irregular masses or chromatin knots but are readily distinguished from them. As the nucleus grows larger the chromatin knots become more widely separated, but synapsis does not occur until it has reached its full size.

During synapsis the reticulum becomes transformed into a definite spirem. The fibers connecting the chromatin knots increase in length and become more uniform in thickness while the knots become less conspicuous as though their material were drawn out along the fibers. The fibers of the reticulum are now seen to arrange themselves in pairs and a general contraction of the network takes place which is the beginning of synapsis. Allen believes that this contraction is associated with the approximation of the fibers. The contracting network occupies at first the center of the nucleus but later moves to the periphery where the nucleoli may be found flattened against the membrane. There is now a continuous spirem in the nucleus, plainly composed of two slender threads lying side by side and probably with no free ends. These two threads often run closely parallel, sometimes loosely twisted about one another, sometimes in contact and apparently fused and sometimes rather widely separated. It is clear that the double nature of the thread is not due to a fission but that two independent threads are developed indepen-

dently out of the reticulum. The two threads gradually fuse so that in older stages of synapsis the nucleus appears to contain a single relatively thick spirem which is shorter and more loosely coiled than in the earlier stages. The minute structure of the threads of the spirem can be determined by careful staining. They consist of a series of chromatin granules (chromomeres) imbedded in the ground substance, linin. As the two threads fuse the chromomeres generally come together in pairs and unite to form a single row of large chromomeres which project from the side of the larger single (fusion) spirem.

The single (fusion) spirem on emerging from synapsis becomes uniformly distributed throughout the nucleus. There appear to be no free ends in the much convoluted and looped thread. Some of the loops become fastened to the periphery of the nucleus but there is no regularity in the number of loops and no relation to the number of chromatic segments that are formed later. While thus evenly distributed the single spirem undergoes a longitudinal fission which is preceded by the division of each chromomere. This is the first longitudinal fission of the spirem which is well known through the descriptions of Guignard, Grégoire, Strasburger, Mottier, and others. The fission is not simultaneous throughout the length of the spirem, for some portions remain undivided for some time when contiguous parts are plainly split. The result is a condition very similar to that presented just before the fusion of the two systems of threads during synapsis which produced the single (fusion) spirem. It seems probable that the threads which become separated are morphologically the same as those which fused during synapsis although the union at that period seems complete. The split spirem remains uniformly distributed throughout the nucleus exhibiting, however, a tendency to become somewhat massed in the center of the nuclear cavity leaving fewer loops attached to the nuclear membrane.

The split spirem now segments throughout its length into the reduced number of chromosomes (12) characteristic of the heterotypic mitosis. The segmentation is not simultaneous, but the first free ends appear near or at the periphery of the nucleus where the split spirem breaks apart at the loops. As segmen-

tation proceeds the number of loops becomes fewer and the free ends more numerous. Allen finds the breaking apart of the arms of the loops, whose heads are at the periphery of the nucleus, as described by Schaffner ('97) and Farmer and Moore (:05), but cannot accept the interpretation of these latter authors (to be described presently). Allen's studies show that the loops are the points of separation of adjacent split chromosomes and not a point where the spirem bends on itself to form a pair of chromosomes. The ends of the split chromosomes when properly stained are seen to be distinct even though they may be in contact or apparently fused. At the time of the segmentation of the split spirem the two threads are generally twisted about one another.

The split chromosomes now shorten and thicken, the number of twists is reduced and the pairs of elements take on the many forms characteristic of the heterotypic mitosis and described as I's, J's, X's, Y's, V's, and O's. These chromosomes of the heterotypic mitosis are of course pairs of chromosomes, *i. e.*, bivalent chromosomes or dyads. They are believed to represent morphologically the full number of sporophytic chromosomes (24) now associated in pairs forming the reduced number (12) of bivalent chromosomes. The two threads which fuse are believed to represent two spirems of maternal and paternal origin and the chromosomes in the pairs are derived from different parents.

Shortly after the segmentation of the spirem the sporophytic chromosomes of each bivalent element or dyad may show evidence of a second longitudinal fission, first recognized by Grégoire ('99), Guignard ('99), and Strasburger (:00) which is completed during the metaphase of the heterotypic mitosis. The evidence consists in the appearance of a double row of granules in each sporophytic chromosome, the result of the division of the chromomeres. However, these chromomeres soon become indistinguishable from the linin and the chromosomes appear homogeneous from now on.

While the spindle of the heterotypic mitosis is being organized the position of the sporophytic chromosomes shifts with the development of the spindle fibers until they are brought to the

nuclear plate still grouped in pairs as dyads (bivalent chromosomes). The details of spindle formation and the heterotypic mitosis do not concern the present discussion of reduction phenomena. The reduction has occurred with the formation of the dyads and the mitosis simply distributes the 24 chromosomes (generally called daughter chromosomes) which are believed to be the morphological equivalents of the sporophytic chromosomes that entered the spore mother-cell from the archesporium.

Just before the separation of the sporophytic chromosomes during metaphase of the heterotypic mitosis a longitudinal fission appears suddenly in each element extending almost the whole length. This is the second longitudinal fission as interpreted by Grégoire ('99), Guignard ('99), Strasburger (:00), Mottier (:03), and others, with whom Allen is in full agreement. It is of course a premature division of the chromosomes preliminary to the homotypic mitosis. The second fission is probably completed at this time but the elements of each pair (formerly called granddaughter chromosomes) remain clinging together at one end by a peculiar overlapping of the hooked tips forming thus a V-shaped pair whose apex is drawn to the poles of the heterotypic spindle. The daughter nuclei following the heterotypic mitosis are not in a true resting condition and the chromosomes while forming a spirem show abundant evidence of independent structure. They emerge from the spirem at the prophase of the homotypic mitosis as the same morphological entities (*i. e.*, as V-shaped pairs) and are thus brought to the nuclear plate from which they are distributed generally as fairly straight rods to form the nuclei of the pollen grains.

Rosenberg's (:03a, :04a, :04b) studies on the hybrids of *Drosera* furnish further evidence that the chromosomes from different parents fuse in pairs during the prophase of the heterotypic mitosis. The gametophyte number of chromosomes in *Drosera rotundifolia* is ten and in *D. longifolia* twenty and those of the former species are larger than those of the latter. The sporophyte number in the hybrid is thirty as would be expected. At the heterotypic mitosis of sporogenesis, however, twenty chromosomes appear in the hybrid, half of which are plainly double structures and consist each of a larger and a

smaller element. During this mitosis the ten double chromosomes divide but the single chromosomes remain entire and either pass to one pole or the other or are left out in the formation of the daughter nuclei. The explanation of these conditions must be that ten chromosomes of *D. rotundifolia* fuse with ten from *D. longifolia* leaving ten of the latter without mates. Rosenberg's last paper (:04b) on *Drosera* describes in considerable detail the union of chromosomes in pairs in both species of *Drosera* during sporogenesis. The sporophytic chromosomes which at first are scattered throughout the nucleus in the early prophase of the first mitosis come together in pairs and unite so closely that there is hardly a trace of their dual nature in the resultant larger bivalent chromosomes, which are of course the gametophyte number. Rosenberg is very positive that the pairs of chromosomes are preliminary to a fusion and not the result of a fission of already reduced segments of a spirem thread. Rosenberg believes that the two halves of the bivalent chromosomes are separated in the first (heterotypic) mitosis and that each splits lengthwise prematurely during the first mitosis in preparation for the second. The fused bivalent chromosomes then appear to divide twice longitudinally but the first division may be only a separation of the two sporophytic chromosomes that entered into the fused pair.

We shall consider now the conclusions of Berghs and Grégoire of the Carnoy Institute, Louvain, whose publications have appeared practically simultaneously with some of those which we have just discussed. Berghs has published three papers (:04a, :04b, :05) treating of the early history of sporogenesis in *Allium*, *Lilium*, and *Convallaria*, and concludes from a study of synapsis that the spirem immediately preceding the heterotypic mitosis arises from the close association, side by side, of two delicate threads. These threads are organized previous to and during synapsis and their coming together brings about that contraction of the chromatic material characteristic of synapsis. The threads contain sporophytic chromosomes of the last mitosis in the archesporium. The apparent longitudinal fission of the spirem which precedes the heterotypic mitosis in the spore mother-cell is interpreted as being these two threads



which are believed to have never actually fused during synapsis. The reduced number of segments derived from the spirem preceding the heterotypic mitosis are then bivalent chromosomes composed of pairs of sporophytic chromosomes lying side by side. The heterotypic mitosis distributes the sporophytic chromosomes in two sets resulting in a numerical reduction of their numbers by one half. It will at once be noted that while Berghs and Allen have independently arrived at similar conclusions respecting the structure of the chromosomes of the heterotypic mitosis there are some important differences in the mode of origin. Allen reports an actual fusion of the two threads (paternal and maternal) during synapsis and a later fission of the spirem previous to the heterotypic mitosis. But the accounts of both authors have much in common in their interpretation of the structure of the spirem and chromosomes of the heterotypic mitosis which is fundamentally different from the accounts of Farmer and Moore, and Strasburger to be described later.

Grégoire (:04) in a general discussion of reduction phenomena confirms the observations of Berghs and takes a very positive position against the interpretations of Farmer and Moore and Strasburger. The chief features of his conclusions are in harmony with the results of Allen. The sporophytic (somatic) chromosomes are believed to become associated in pairs by the application of two delicate threads throughout their length during synapsis. These threads are believed to retain their autonomy and never actually to fuse although they may come in close contact. Consequently the reduced number of chromosomes are pairs of sporophytic chromosomes which have retained complete independence. Allen, on the contrary, reports a complete union of the two threads involving the fusion of chromomeres in pairs and a later longitudinal division throughout its length of the single (fusion) spirem. Grégoire does not regard the heterotypic mitosis as a true nuclear division but as a special process designed to effect this numerical separation of the sporophytic chromosomes and intercalated between typical mitoses, while Allen would apparently treat it as a true mitosis and regard the chromosome reduction as effected by the fusion of two sporophytic spirems during synapsis.

Rosenberg (:05) has recently published a general review of reduction phenomena based on studies upon *Listera*, *Tanacetum*, *Drosera*, and *Arum*, taking a position in essential agreement with Allen and the investigators of the Carnoy Institute and in opposition to the theory of Farmer and Moore and Strasburger. Rosenberg does not quote Allen's preliminary paper (:04) which anticipates his conclusions. He finds that the spirem which emerges from synapsis is preceded by a condition when the structure is clearly made up of two threads (spirems) which lie parallel to one another. These two threads are frequently joined together, and in places spirally twisted but here and there they may be seen to be entirely separated from one another. They finally form the single spirem which follows synapsis and which divides into the reduced number of chromatic segments. But the chromatic segments throughout the entire processes are shown to be double in structure (bivalent chromosomes), *i. e.*, composed of two chromosomes lying very close together side by side or even united. What appears to be a longitudinal fission of the chromatic segments of the spirem immediately preceding the first mitosis is really then a line of union along which the two independent threads have come together. The phenomenon of synapsis consists of this close association of two threads which are themselves simple spirems into a double spirem which segments into pairs of sporophytic chromosomes each of which may be regarded as a bivalent chromosome.

Farmer and Moore published a preliminary communication in 1903 which aroused much interest in their theory of chromosome reduction. The full account (:05) has recently appeared. Their studies are upon *Lilium*, *Osmunda*, *Psilotum*, *Aneura*, and the cockroach, *Periplaneta*. *Lilium* and *Osmunda* among the plants were given chief attention and since the lily was the type studied by Allen it will serve best to contrast the conclusions of these two investigators. The accounts of Allen and Farmer are so fundamentally different as regards the events of synapsis and the prophase of the heterotypic mitosis that it seems scarcely possible that both can be right in their respective material, *Lilium candidum*, Farmer's type, and *L. canadense* of Allen's description. Farmer and Moore intro-

duce the terms “maiosis” and the “maiotic phase” to cover the whole series of nuclear changes included in the heterotypic and homotypic mitoses. The maiotic phase is regarded as similar in its essential details in both animals and plants but the fact of its appearance at different points in the life histories precludes any probability of relationship in such widely divergent lines. The events of synapsis and the consequent peculiarities of the heterotypic and homotypic mitoses are considered as intercalated between the series of typical mitoses in the life history.

Farmer and Moore's conclusions for *Lilium candidum* may be briefly summarized as follows. A definite spirem with the chromatin distributed as granules appears in the young spore mother-cell before its separation from neighboring elements. A “first contraction figure” now appears and the spirem thread becomes densely coiled in the vicinity of the nucleolus, this condition persisting for some time. Then the coils of the spirem loosen and become distributed about the periphery of the nuclear cavity, from the point of contraction as a center. A longitudinal fission of the spirem thread then appears, the chromatin granules dividing so that they come to lie in two parallel rows on the edge of the split ribbon. The fission is irregular and open loops appear at places. The spirem then shortens and the split gradually closes up and becomes very difficult to recognize. Many of the convolutions of the thread are attached to the nuclear membrane while the remainder form a tangle in the interior around the nucleolus which is believed to give up much of its substance to the chromatic portion of the spirem. Farmer and Moore then fail to find the double thread and its union during synapsis to form a single (fusion) spirem which is a fundamental feature of Allen's account.

There follows then a stage which has been the subject of much discussion. According to Farmer and Moore the spirem thread becomes pulled out into V- and U-shaped loops, shown with especial clearness where the bend of the loop is attached to the periphery of the nuclear membrane. The arms of the V's then come to lie parallel and so close together as to give the appearance of a fission in a structure which is really the result of an approximation of the two free ends of what was a loop.

The spirem thread thus breaks up into segments which, however, lie in pairs represented by the V's in the reduced (gametophyte) number. The pairs are bivalent chromosomes, each composed of two sporophytic chromosomes which were arranged serially on a single spirem thread. The pairs are not always organized through the approximation of the arms of V-shaped loops but this is a very characteristic type of structure. The V's have been interpreted by other authors as the approximation of portions of the spirem thread (Dixon, '95, '96, :00) or the separation of their free ends at the bend of the loop as a transverse division of a reduced number of looped chromosomes in the heterotypic mitosis (Schaffner, '97). The two parts of the bivalent chromosomes (which are pairs of somatic chromosomes) now become shorter and thicker and all trace of the original fission of the spirem thread is lost.

The essential features of Farmer and Moore's interpretation of the prophase of the heterotypic mitosis are, then : (1) a single spirem with the sporophytic chromosomes arranged serially, which splits only once longitudinally, the fission afterward becoming obliterated when the chromosomes are organized, and (2) the organization of bivalent chromosomes in the reduced number largely by the approximation of the free ends of loops which entails a separation at the bend of the loops of the two sporophytic chromosomes, giving the appearance of a transverse division.

The heterotypic mitosis, then, according to Farmer and Moore involves merely the distribution of the sporophytic chromosomes arranged in pairs (bivalent chromosomes) as univalent elements to each daughter nucleus. This is of course the general conclusion of all recent investigators, the different views being the result of varying accounts of the method of organization of the bivalent chromosomes. During this distribution in the heterotypic mitosis the split of the original spirem appears in each univalent element (sporophytic chromosome) and the halves open throughout the greater part of their length giving the peculiar V-shaped daughter chromosomes so characteristic of this mitosis in the lily. The arms of these V's become the daughter chromosomes of the homotypic mitosis which are thus

formed prematurely during the heterotypic as was first described by Grégoire ('99). However, Grégoire and most botanists have considered the split between the V's as a second longitudinal fission of the original spirem in the spore mother-cell while Farmer and Moore regard it as the reappearance of an original single fission. This view of Grégoire, which has had the support of Guignard ('99), Strasburger (:00), and Mottier (:03), is the theory of a double longitudinal splitting of the chromosomes previous to the heterotypic mitosis and is also maintained in Allen's (:05) recent paper.

The homotypic mitosis brings about the final separation of the arms of the V-shaped longitudinally split univalent (sporophytic) chromosomes of the heterotypic division. The fact that the arms of these V's finally break apart at the ends does not constitute a transverse division as has been claimed by some earlier writers (Ishikawa, '97; Calkins, '97; Belajeff, '98; Atkinson, '99, for Trillium). The peculiarities of the homotypic mitosis are then due to the premature fission of the univalent chromosomes during the heterotypic. As a type of nuclear division the homotypic mitosis is not fundamentally different from the typical divisions of other periods of the life history. All recent authors are in agreement on this interpretation of the events of the homotypic mitosis.

Gregory (:04) gives an account of sporogenesis for several leptosporangiate ferns and accepts Farmer and Moore's explanation of reduction phenomena. He finds the same sort of U-shaped segments in the reduced number at the heterotypic division and considers them bivalent chromosomes which divide transversely so that the original sporophyte chromosomes are distributed in two sets during this mitosis. The various positions assumed by the limbs of the U-shaped segments give appearances very similar to the tetrads described in the heterotypic mitosis of animals and which Calkins ('97) reported for *Pteris* and *Adiantum* and regarded as resulting from the transverse division of the halves of a longitudinally split chromosome. Gregory of course cannot accept the conclusions of Calkins.

Williams (:04a) applies the theory of Farmer and Moore respecting the bivalent character of the chromosomes in the

heterotypic mitosis to his studies on the first division in the tetraspore mother-cell of Dictyota. But it can hardly be said that his account offers any material support to the theory. There is a clear synopsis stage preceding the mitosis in this form from which a spirem emerges as a beaded thread. This spirem then becomes split longitudinally and later the chromosomes are organized and show a longitudinal fission. The form of the chromosomes at metaphase of the first mitosis is heterotypic, a ring form being prevalent, and Williams concludes that it is developed by the bending and closing of the free ends of a loop. The events of synopsis are not clearly enough known to make possible a comparison with the accounts of Allen and Berghs.

We are now ready to take up the latest conclusions of Strasburger (:04b) which are closely associated with views expressed in a recent paper of Lotsy (:04). Lotsy gives a clear statement, illustrated with many diagrams of the various ways in which sporophytic chromosomes may be conceived to unite in pairs previous to the first mitosis in the spore mother-cell and the manner in which the resultant bivalent chromosomes may be divided and distributed by the two mitoses of sporogenesis. Lotsy makes parallel comparisons between sporogenesis in plants and gametogenesis in animals and proposes the term "Gonotokonten" ("Nachkommenbildner") for the mother-cells which inaugurate reduction phenomena. The paper presents no new observations but discusses the problems of reduction in their broad aspects. An excellent summary is given by Koenicke (:04).

Strasburger's (:04b) most recent paper, "Ueber Reduktionsteilung," is based chiefly on studies of Galtonia and Tradescantia and presents an entire change of view from his conclusions of 1900. Galtonia seems to be a very favorable form for study since the gametophyte number of chromosomes is only six and the structures are exceptionally clearly differentiated in the spore mother-cells, which Strasburger calls "Gonotokonten" after Lotsy. A single spirem is reported to split longitudinally but the two daughter threads remain close together. The spirem then shortens and thickens and becomes distributed in heavy

loops. It finally divides into six segments which are interpreted to be six pairs of chromosomes joined end to end. These six segments are then bivalent chromosomes. The two chromosomes of each pair (segment) finally come to lie side by side in various positions by the bending of the original looped segments and the separation of their two ends in the middle. The halves of the six bivalent chromosomes (segments) are distributed by the first mitosis so that there is the effect of a transverse division of six chromosomes at this time, but really the process is one of the distribution of twelve chromosomes in two sets of six each. The longitudinal fission of the spirem thread becomes more conspicuous towards the end of the first mitosis so that the twelve chromosomes become partially split and pass as V's to the poles of the first spindle during telophase. This premature division is preparatory for the second mitosis (homotypic) when the separation is finally effected. There is then only one longitudinal fission of the original spirem in the spore mother-cell and this prepares the chromosomes for the second mitosis, which differs only from the typical mitoses in the premature splitting of its chromosomes. The first mitosis is merely the separation of pairs of chromosomes joined end to end. Strasburger interprets the conditions in *Tradescantia* and *Lilium* in a similar way believing that the complications there simply arise from a more involved looping of the spirem thread. Strasburger's account of *Galtonia* then supports in all essentials the theory of Farmer and Moore.

Strasburger in the same paper (:04b) gives an account of synapsis which cannot be brought into harmony with that of Allen. The chromatin granules are reported to gather during synapsis into as many centers, which he names "*Gamozentren*," as will finally form the reduced number of bivalent chromosomes (six in *Galtonia*). The "*Gamozentren*" then become arranged and drawn out into the spirem which emerges from synapsis. The chromatin granules are named "*Gamosomen*" and the bodies formed in the "*Gamozentren*" which afterwards become the bivalent chromosomes of the first mitosis are called "*Zygosomen*." There are then no organized chromosomes during synapsis and no place in Strasburger's account for the fusion of

a fully organized paternal and maternal spirem as described by Allen. The identity of the sporophytic chromosomes becomes entirely lost, according to Strasburger's explanation of synapsis, and the chromatin granules ("Gamosomen") may be variously distributed in the new set of bivalent chromosomes ("Zygosomen"). These "Zygosomen" are a new creation in the cell. All of the other theories, on the other hand, preserve the morphological entity of the sporophyte chromosomes which are of course of maternal and paternal origin but allows their distribution in various ratios to one another during the first mitosis of sporogenesis. The chromosome, however, remains a fixed morphological structure from one generation to another. These are fundamental differences which have a vital bearing on the discussion of hybridization, which will follow shortly, since one of the most important features of the problems concerns the preservation of the relative purity of the germ plasm.

The chief characteristics of the two theories of reduction may be summarized as follows:—

(1) According to Allen, Rosenberg, Berghs, and Grégoire, the phenomenon of synapsis presents a close association of two parallel chromatic threads (probably of maternal and paternal origin) which finally unite to form the spirem that precedes the heterotypic mitosis. This single (fusion) spirem is then double in nature and the longitudinal fission which follows, is the separation of the two threads that entered into its composition. The reduced number of chromatic segments of the heterotypic mitosis are bivalent chromosomes or more precisely pairs of sporophytic chromosomes derived from the two (maternal and paternal) threads of the synapsis stage. The heterotypic mitosis distributes the sporophytic chromosomes in two sets thus effecting a numerical reduction by one half. The sporophytic chromosomes divide prematurely during the heterotypic mitosis in preparation for the homotypic thus presenting a second longitudinal fission of the segments derived from the single (fusion) spirem. A special feature of Allen's studies is the fusion of chromomeres in pairs during the organization of the single (fusion) spirem and a subsequent splitting of each larger chromomere with the longitudinal fission of this structure.



(2) Farmer and Moore, Gregory, Williams, and Strasburger hold that there is primarily only a single chromatic thread in the nucleus of the spore mother-cell which is the spirem of synapsis and the heterotypic mitosis and which most of these authors believe to be composed of the full number of chromosomes (sporophytic) joined end to end. This spirem splits longitudinally but the fission is a premature division which prepares the chromosomes for the homotypic mitosis. The chromosomes of the heterotypic mitosis are formed from loops of the spirem which include a pair of sporophytic chromosomes joined end to end. The members of this pair come to lie side by side by an approximation of the arms of the loops and a breaking apart at the head of the structure. This transverse fission of the spirem is not of course a transverse division of a chromosome but merely the separation of a pair of chromosomes joined end to end. The line between the two arms of the loop marks a region of contact due to approximation and not a line of fission. The heterotypic mitosis effects a numerical reduction of the chromosomes as in the first view but these chromosomes are formed on entirely different principles. A single premature fission of the spirem or its segments prepares the chromosomes for the homotypic mitosis.

Comparing the two schools, it may be noted that they both explain reduction phenomena as a numerical reduction of the double set of sporophytic chromosomes by a distribution in two sets. The fission of the chromosomes is always quantitative and there is no hint in any of the views of a qualitative division in Weismann's sense. Furthermore, most of the investigators are firmly convinced of the individuality of the chromosomes which means that they are convinced as morphological entities persisting from one generation to the next. This is an important agreement in relation to theories of heredity and hybridization which we shall discuss at another time (see treatment of "Hybridization"). The differences lie in questions of fact regarding the organization of these chromosomes in the spore mother-cell and their behavior during synapsis and at other periods of prophase in the heterotypic mitosis. There is entire accord in that the chromosomes of the homotypic mitosis appear

during the metaphase of the heterotypic but a fundamental difference in the accounts of the manner in which these structures are formed.

In conclusion, we may very briefly note the fact that the zoölogists are divided into two schools in their accounts of reduction phenomena, apparently along similar lines to those of the botanists. Some recent papers (Winiwarter, :00; Schoenfeld, :01; and the Schreiners, :04) have described the union of parallel threads (maternal and paternal) during synapsis to form a single spirem in the rabbit, man, bull, hag-fish, and shark. Winiwarter and the Schreiners regard a later longitudinal fission of the spirem as a separation of the two threads which originally entered into the structure. The chromosomes in the hag-fish (*Myxine*, the Schreiners, :04) are organized in pairs side by side and a second longitudinal split appears in each. The heterotypic mitosis separates the groups in the plane of the first fission and the two parted chromosomes are divided by the homotypic. This history is essentially similar to Allen's account of the lily. On the other hand there is a large body of observations founded on the investigations of Häcker, vom Rath, Rückert, Montgomery, and others, indicating that bivalent chromosomes are formed consisting of somatic chromosomes joined end to end and that these elements or their derivatives are distributed either with the heterotypic or homotypic mitosis. This of course involves a transverse division which is, however, interpreted as the separation of adjacent chromosomes and not as a qualitative division in Weismann's sense. The attitude of the first group is clearly similar to that of Allen, Rosenberg, Berghs, and Grégoire among the botanists, while that of the second shows many points of similarity to the theory of Farmer and Moore and to Strasburger's last view (:04). There are a number of accounts of a double longitudinal fission of chromosomes especially among the vertebrates, which have not been harmonized with the last view but may find explanation along the lines of the more recent investigations.

It is of course conceivable that there are two distinct types of arrangement of sporophytic and somatic chromosomes in animals and plants at synapsis during gametogenesis and sporogenesis.

It is possible that they may be grouped in pairs (bivalent chromosomes) either side by side through two parallel threads (paternal and maternal spirems) or end to end in a single chromatic thread. But it will certainly be interesting if animals and plants both show variations in these respects and very remarkable if the same genus, as *Lilium*, should present contrasting types of reduction phenomena. And on these points must be concentrated the future investigations in this field.

While we are making progress in our understanding of the behavior of the chromosomes it must never be forgotten that in them we are dealing only with the most conspicuous form of germ plasm and that there are much finer elements which in their turn will demand attention. We may hold to the view of the individuality of the chromosomes as morphological entities but nevertheless we must recognize the fact that the substance of these bodies which stand for parental characters, the idioplasm of Nägeli, may pass through remarkable changes which are far from understood. There is much evidence that the parental idioplasm may mix or combine during synapsis in the organization of the spirem from which are developed the reduced number of bivalent chromosomes. Allen has described the actual fusion of sets of chromomeres believed to be of maternal and paternal origin and there are many possibilities of the two idio-plasms reacting upon one another to bring about intimate and fundamental interrelations. These become important principles in discussions of heredity and hybridization and will be considered later. Allen (:05, pp. 246–252) presents an admirable analysis of these problems.